RB
132
N.S.
v.2
no.4
Biological
& Medical
Serials

New Series, Vol. II. No. 4.

PROCEEDINGS

OF THE

NEW YORK PATHOLOGICAL SOCIETY

LIBRARY
UNIVERSITY OF TORONTO

May, 1902.

PUBLISHED BY THE SOCIETY

AUGUST JEROME LARTIGAU, Editor
437 West 59th Street, New York City

Proceedings

of the

New York Pathological Society.

New Series, Vol. II. MAY, 1902. No. 4

TABLE OF CONTENTS.

Jessup, Primary Carcinoma of the Vermiform Appendix.—Ewing. Vaccine Bodies in Variola.—Williams, Studies on the Etiology of Vaccinia and Variola.—Park, Preliminary Report of Experiments with Filtered Vaccine. — Ewing, The Relation of Streptococcus Pyogenes to Variola.—McGill, The Chemical Theory of Small-pox.—Pollitzer, Demonstration of the Skin Lesions of Small-pox.

Dr. James Ewing, *President.*

PRIMARY CARCINOMA OF THE VERMIFORM APPENDIX.

D. S. D. JESSUP, M.D.

The specimen was removed at the Woman's Hospital in April, 1902, by Dr. Clement Cleveland. The patient was a woman, thirty-six years old, who had had two children and five abortions. Since the last abortion she had suffered with pain in the left inguinal region and the operation was undertaken for disease of the uterine adnexæ. Abdominal incision showed a cyst of one ovary, which was opened. The appendix was bound down with adhesions and was therefore removed. The patient made an uneventful recovery.

The gross examination shows an appendix six cm. long. At the junction of the middle and outer thirds it is bent at a right angle. There is a constriction at the

bend and beyond this a dilatation giving a diameter of one cm. The proximal two-thirds are five mm. in diameter. The peritoneal coat appears smooth, although adhesions were present at the time of the operation. Section shows thickening of the wall in the proximal two-thirds, leaving a narrow lumen here. At the bend the lumen is obliterated. In the dilated portion the muscular coat appears as a thin shell and the space within is occupied by a tumor mass of firm, yellow tissue.

Microscopical Examination: Section of the tumor shows an adeno-carcinoma. The new growth has replaced the structures of the mucosa and submucosa, and its cells are infiltrating the muscular coat. The cells are grouped in alveoli of various sizes and shapes and between the alveoli is a small amount of muscular and connective tissue. In the alveoli which show a lumen, the cells are cylindrical in shape. in other places they are polygonal. The middle and proximal portions of the appendix are not invaded by the new growth, but there is a marked activity of the germ centers of the lymph nodules.

Apart from the rarity of this condition the case is of interest on account of the absence of any symptoms pointing to disease of the appendix so that its discovery was an accidental one.

A number of cases in which carcinoma of both appendix and cæcum have been found have been reported, and Kelly (1) has collected seventy-nine such cases. These, however, cannot be considered in determining the frequeucy of primary carcinoma of the appendix for there was no evidence to show that the growth originated in the appendix. Other cases of carcinoma confined to the appendix alone, I have not included in the following list because the diagnosis appears to have been made

on gross examination alone. The thirteen cases collected are:

1. Reported by Beger (2). The carcinoma of the appendix was in a man forty-seven years old and was found in an operation on a sinus following drainage of an abscess in the right iliac fossa. At autopsy the abdominal lymph nodes were found to be invaded.

2. Reported by Stimson (3). The appendix, removed at operation from a woman forty-four years old, was four inches loug and in a state of "cancerous degeneration." She had had two attacks of appendicitis, the first ten years before.

3. Mossé and Daunic (4) report the case of a woman, fifty years old, who died of heart disease. The appendix, removed at autopsy, was four cm. long and four and five-tenths cm. in circumference. The new growth occupied the whole length of the organ. There was no history of appendicitis.

4. Wright's (5) case was a patient dying of general purulent peritonitis. A perforation was found at the junction of the cæcum and appendix and a carcinoma at the tip of the appendix.

5. Of two cases reported by Kelly (1) the first is without clinical history. The appendix, removed at operation, was eleven cm. long and the tumor was located in the distal portion.

6. The second case was a man, nineteen years old. An operation was performed nine days after the first symptoms of appendicitis appeared. The appendix was found surrounded by a small collection of pus. It measured five cm. in length and the tumor was situated at the base.

7. The case described by Elizabeth Hardon (6) was that of a woman, twenty-four years old. There was a

history of pain in the right iliac fossa. At operation adhesions were found enveloping the appendix. It was ten cm. long and the tumor occupied the middle portion.

8. Rolleston (7) reports an appendix removed by operation from a woman twenty-six years old, who was suffering from her fourth attack of appendicitis. The tip of the appendix was adherent to the uterus and the tumor was located here.

9. Whipham's (8) case was removed at autopsy, from a woman forty-five years old. There was no history of appendicitis and death was due to emaciation and exhaustion caused by the abdominal condition. There was a carcinoma of the base of the appendix and secondary deposits in the liver, ovary, lymph nodes, and peritoneum.

10. Of two cases reported by McBurney (9), the first was a woman twenty-three years old, operated on after a second attack of appendicitis. The appendix was four inches long and the tumor, the size of a pea, was located at the apex.

11. The second case was a man of about thirty years who gave no history of appendicitis, At autopsy, Lartigau found a carcinoma of the tip of the appendix.

12. The case operated on by Goffe (10) was a girl of fifteen years, who had had symptoms of trouble with the appendix for one year. The tumor was located at the tip.

13. Harte and Wilson (11) report a carcinoma of the middle third of the appendix. It was removed by operation from a woman twenty-four years old who gave a history of appendicitis.

The type of carcinoma in the cases where it is described is adeno-carcinoma. Nine of the cases are in women and three in men, and the fact that in most of

the cases the condition is unsuspected will tend to maintain its greater frequency in women because they are more often subjected to abdominal section.

It is noteworthy that two of the cases are under twenty years of age and four under thirty years. There was a history of appendicitis in seven of the cases and no history of it in five. The location of the tumor is most often at the apex, being here in eight instances.

In four cases the new growth was found at autopsy and in the remaining cases at operation, but in no case had carcinoma of the appendix been previously diagnosed.

Routine microscopical examination of all appendices removed will doubtless in the future show that carcinoma occurs in the appendix more often than has hitherto been thought.

References.

1. Kelly: Proc. Path. Society, Philadelphia, N. S., Vol. III, p. 109.
2. Beger: *Berliner klin. Wochenschrift.* 1882—19—616.
3. Stimson; *Annals of Surgery.* 1896, XXIII, 186.
4. Mossé et Daunic: *Bulletin de la Societe anatomique de Paeis.* 1897, LXXII, 814.
5. Wright: *Boston Med. and Surg. Journal.* 1898, CXXXVIII, 150.
6. Hurdon: *Johns Hopkins Hospital Bulletin.* XI, 175.
7. Rolleston: *Lancet:* 1900, II, p. 11.
8. Whitham: *Lancet.* 1901, I, p. 319.
9. McBurney: *Medical Record.* 1901, LX, p. 478.
10. Goffe. *Medical Record.* 1901, LX, p. 14.
11. Harte and Wilson: *Medical News.* 1901, LXXVIII, p, 389.

VACCINE BODIES IN VARIOLA.

JAMES EWING, M.D.

The paper is a review of the results of bacteriological examination by ordinary methods of cadavers of twenty-nine cases of variola.

Vaccine bodies were studied in the cutaneous and visceral lesions of variola by a variety of methods.

In the fresh condition the fluid from the cutaneous lesions at various stages was examined, unstained, and stained by gentian violet. By this method many leucocytes and epithelial cells and many red cells may be distinguished and set aside.

The sporocysts described by Funck (*British Med. Journal*, 1901, I, p. 448), seemed to correspond to certain large cells containing one or many greenish refractive globules. In stained smears these resolve themselves into vacuolated leucocytes and epithelia. Myriads of fine granules are usually present which stain like protoplasmic detritus.

The typical vaccine bodies are larger, spherical. homogeneous, or translucent bodies, colorless or yellowish, with or without membranes, from 1 to 7 micra in diameter. Many are extra-cellular, some within other cells. Treated with gentian violet the membranes about many of them become distinct.

By this and other staining re-agents the bodies exhibit certain polychromatic tendencies varying toward distinct basophilia. The resemblance of many of the polychromatic bodies to the red cells in the pustules which were undergoing subdivision with loss of distinct acidophile staining suggested that many of the vaccine bodies might be derivatives of red cells. The bodies with membranes and the small crescentic forms, especially, seemed to have this origin. In stained specimens some of the bodies stain blue, some red, some red on the periphery and blue in the center. These latter forms resemble the polychromasia which Maragliano described in circulating red cells.

I failed to find any traces of ameboid movements in many specimens examined on a warm stage.

In sections of the skin and mucous membranes, the

main fact elicited was that the vaccine bodies are usually present in the lesions in all stages up to complete healing. They were not always present in the early necrotic foci of the tracheal and esophageal mucosa. The forms of the bodies encountered included all of those described at length by Hückel.

The bodies appeared in large numbers in the skin and mucous membranes in the petechial spots of a case of variola with ulcerative endocarditis dying in the third week of the disease. In some of these spots, however, only fragmented acidophile red cells appeared. They were unusually abundant in a case dying in the late pustular stage in which fresh hemorrhage occurred into the pustules (black smallpox). The abundance of the bodies seemed to bear a constant relation to the severity of the disease, and especially to the hemorrhagic character of the lesions. In the well-developed early pustule they were most numerous in the large flat cells on the outskirts of the necrotic zone. In the necrotic zone they were less numerous, were often found disintegrating and granular, and distinguished with difficulty. They were seldom numerous in the foci exhibiting most active degeneration. In the early stages of necrotic lesions of the trachea, many foci were found in which vaccine bodies were absent. The impression was therefore gained that if the variolous poison comes from these bodies it is distributed in a most erratic manner. Infected cells must certainly long remain alive, and in some healing lesions it seemed equally certain that many infected cells would not suffer permanently from the presence of the body. In necrosing cells the bodies were found to vary in size, to appear as rosettes, crescents, rods, and finally to break down into fragments, sometimes with vacuolation, along with the cytoplasm of the harboring cell. Sometimes

the necrosing cells become condensed and present an appearance re-calling that of some sporocysts. In the necrosing epithelium of the cornea, conjunctiva, and nictating membrane of healthy dogs, I failed to find any structures resembling vaccine bodies.

The distribution of vaccine bodies was found to be co-extensive with the lesions of the disease, whether these were of the strictly variolous focal type or induced by secondary bacterial invasions. They were found in the cutaneous lesions of the pharynx, epiglotic folds, in the ulcerated nasal and ethmoidal sinuses, very abundantly in the trachea and bronchi, in the esophagus, in circumscribed or diffuse ulcerations of the stomach, abundantly in the intestine, and in the renal pelvic epithelium.

I examined various lesions of the skin and mucous membranes to determine if vaccine bodies are present in other than variolous processes, and while never finding in any other lesions great abundance of bodies resembling the vaccine corpuscles, I found a scanty or moderate number of structures indistinguishable from vaccine bodies in streptococcus inflammation of the skin, in erysipelas, in pemphigus, in dermatitis herpetiform, in purpura hemorrhagica, and in measles,

In the decaying skin of variola, the vaccine bodies disappeared without sign of multiplication between the fourth and eighth days.

THE ORIGIN OF VACCINE BODIES.

Some vaccine bodies seem to give evidence of an origin from the cytoplasm of the epithelial cells. This is especially true of those bodies which are connected

with the cytoplasm of the cell by radiating fibres, and of those forms which exhibit a central basophile mass fringed by a peripheral acidophile portion. Yet the smallest and presumably the earliest vaccine bodies do not exhibit these radiating threads, but are extremely well-defined from the cytoplasm and impress the observer as being distinctly foreign to the cell. Hückel had some difficulty in adjusting his theory of a cytoplasmic origin to the appearance of these small compact bodies.

My belief that many of the typical vaccine bodies in human tissues are remnants of red cells has been reached very slowly. It first appeared in the study of human material that vaccine bodies were most numerous in the hemorrhagic lesions, and that by some stains—*e. g.* Heidenhain's iron-hematoxylon—the vaccine bodies behave very much like red cells. Distinct transitional stages between red cells and fragments and basic vaccine bodies were not encountered until late in the series, when a trachea which had been hardened in Orth's fluid (Müller-Formalin), exhibited a great variety of forms which resembled red cells in their tendency to stain with eosin more than the usual vaccine body.

Likewise, in a late secondary hemorrhagic case, in which recent profuse hemorrhage had occurred into the pustules, a great variety of forms, apparently transitional between red cells and vaccine bodies, were found in and between epithelial cells.

Finally, in the renal pelvic epithelium of two primary hemorrhagic cases was encountered a profuse display of the englobement of red cells and their fragments by epithelia, and their transformation into polychromatic vaccine bodies. Here were found red cells unaltered, within and between epithelial cells—large red cells with beginning or advanced vacuolation, some strongly acido-

phile, some strongly basophile; all sizes of acidophile fragments; some epithelial cells holding within the same vacuole various shades of acidophile and basophile fragments of red cells, englobed red cells with basophile centers and acidophile peripheries, or fragments fringed with protoplasmic or hematoidin granules. These polychromatic qualities were best demonstrated by staining with strong hematein and strong watery eosin, With dilute eosin most of the bodics remained tinged with hematein. By methylene blue or Nocht's method, the bodies usually stained light or dark blue. Their perinuclear position and distinct halo were identical with those of the typical vaccine body of the cornea.

The above observations, which were readily dupliated by a review of the previous material, seem to place beyond donbt the conclusion that red cells may be, and very frequently are transformed into bodies indistinguishable from vaccine bodies.

Yet in specimens fixed by Müller's or Orth's fluid, strong staining in watery eosin develops a certain acidophile tendency in many of them although leaving the nuclei of epithelia and leucocytes uniformly dark blue.

The belief that many vaccine bodies are derived from red cells accords with many facts concerning the occurrence of these bodies and the changes which may affect red cells. Two familiar types of degeneration of red cells occur in the circulating blood in various conditions, especially in cases of severe streptococcus infection. They are first, polychromasia of Maragliano, in which a central basophile portion of the red cell separates from a broad peripheral portion containing all of the hemoglobin remaining in the cell; and second, polychromasia of Gabritschewsky, in which the entire cell stains a brownish color after treatment with eosin and methylene

blue or eosin and hematoxylon. Punctate basophilia and schistocytosis or subdivision of red cells with the appearance of basic centers of the fragments also frequently occur in the circulating blood. A pronounced case of this latter type has been described by K. Ehlich and Lindenthal (*Zeit. f. klin. Med.*, Bd. 30, p. 427), in nitrobenzol poisoning. The basophile portion is regarded as the remnant of nuclear material in the red cell. Now, all of the above changes in the red cells occur in very unusual abundance in and about the focal lesions of variola and vaccinia. In the earlier stages of the lesions the necrosing tissues and their vicinity are literally drenched with fragments of subdivided red cells exhibiting various polychromatic staining qualities. I have seen no other type of lesion in which such active destruction of red cells occurs about necrotic foci.

Hence, about the inflamed cells of the various lesion of epithelium there are a great abundance of already altered fragments of red cells, which, when englobed by the epithelia at once possess the characters of vaccine bodies.

The somewhat specific quality of this destruction of red cells about the variolous lesion may explain the abundance of vaccine bodies in such foci, which is practically a pathognomic feature of variola,

The perfectly homogeneous quality of the majority of vaccine bodies has suggested that they represent a form of hyaline degeneration of the cytoplasm; but the sudden appearance of these compact masses in the cytoplasm of epithelia has distinctly impressed the minds of most observers, including Hückel, with the idea that they must be bodies foreign to the cell. The granular edges of some of the bodies may be referred to the disintegration of the outer rim of the red cell, while the pseudo

pods of vaccine bodies are identical in appearance with the knobs on shrinking and deformed poikilocytes. Sometimes there is a rim about the compact vaccine body such as exists also about hardened and densely stained fragments of red cells. The occurrence of hematoidin granules about the halos of some vaccine bodies is an unusual but unmistakable suggestion of the true origin of these particular individuals.

While it seems certain that many bodies now passing as probable parasites are remnants of red cells, before attempting to extend this theory of origin to all vaccine bodies it is clearly necessary to demonstrate such a relation for the vaccine bodies in the corneal epithelium. Here, in a bloodless tissue, it seems hardly possible that red cell detritus can account for the great numbers and comparatively wide distribution of vaccine corpuscles. Hence, the theory of the existence of a specific form of vaccine body, possibly parasitic, remains almost untouched.

That the vaccine bodies of the cornea are not derived from red cells forced into the epithelium from the lymph spaces communicating with the canal of Schlemm, is practically certain, because these lymph spaces in corneal vaccinia, at least in the centers of the cornea, are usually free from red cells. Another possible mode of origin is from the abundant red cell detritus of vaccine which might account for the bodies seen in one inoculation wound, but Wasielewski claims to have transferred vaccine from cornea to cornea through forty rabbits, finding the vaccine bodies undiminished in each case. These results, if correct, indicate that the corneal vaccine body is either a parasite, or, as Hückel believes, a derivative of the epithelial cell.

Considering the very peculiar conditions of nutrition

of the corneal epithelium pointed out by Ranvier, it may very well be that entirely different processes take place in these cells and in vascular human tissues, and that the vaccine bodies of the cornea are entirely different from those of other tissues. Against this possibility is the very great similarity between all typical vaccine bodies, while the results of the present study still further complicate the subject by indicating that red cell detritus may assume forms apparently indistinguishable in position, morphology, and staining re-actions, from typical vaccine bodies.

Possibly some method may be secured by which one can distinguish red cell detritus from vaccine bodies; or, on the other hand, some facts may be gathered pointing to the possible access of red cell detritus to the corneal ulcer.

STUDIES ON THE ETIOLOGY OF VACCINIA AND VARIOLA.

ANNA W. WILLIAMS, M.D.

The studies reported in this paper may be divided, according to the methods employed, into two parts, first bacteriological and second histological.

The direct object of the bacteriological studies has been to determine if an organism in pure cultures could be obtained which would produce typical vaccinia or variola, while that of the histological studies has been to determine the nature of the "vaccine bodies."

In the bacteriological studies the plan of work was as follows:

1st—Studies of serial and isolated smears and hanging drops from eruptions on rabbits, calf, monkey and

man, from corneas of rabbits, from fresh and old glycerinated vaccine virus and from filtered lymph.

1nd—Cultures made from all of these materials but especially from the inoculated corneas of rabbits and old glycerinated vaccine virus, using ordinary and special media. The ordinary media were used as controls. The special media used were, (1) ascitic-broth, ascitic-broth in celloidin capsules inserted into the peritoneal cavity of the rabbit, (2) wounded peritoneum and subcutaneous tissue of vaccinated and non-vaccinated rabbits, (3) rabbits skin in the peritoneal cavity of the rabbit, (4) foetal rabbits skin taken with aseptic precautions, (5) fertilized hens' eggs.

The most interesting results were obtained from the inoculated rabbit corneas. These experiments are still in progress. The technic is as follows:

The corneas are inoculated with old glycerinated virus and, after a varying time, the animals are killed, the eyes removed under aseptic precautions and washed in sterile broth. The whole cornea is then cut out, washed in successive sterile broths, placed on an agar plate and kept at 36° C. over night. If there is no apparent growth the next day, the cornea is placed in ascitic-broth after smears and hanging drops have been made from the inoculated area, and returned to the thermostat. After 24 hours much of the epithelium has become loosened and on shaking the ascitic-broth becomes slightly cloudy with apparent irregularly granular detritus. Microscopically, besides much finely granular material there are seen a few vaccine-like bodies free and many epithelial cells and leucocytes in various stages of degeneration, some of the former still containing vaccine bodies.

The ascitic broth containing these cells, vaccine

bodies and granules when rubbed on freshly shaved rabbit's skin produces a typical eruption.

The chief points of interest in this cultural study of the corneas are, first, that it has given us a method for obtaining active vaccine virus unmixed with discoverable organisms, and second, that it shows us the vaccinia is not produced by an organism or a combination of organisms growing rapidly in ascitic-broth.

The histological studies were made principally upon rabbits corneas. The sections were prepared by Dr. Flournoy.

Control inocculations were made with (1) inactive vaccine virus, (2) filtered vaccine virus, (3) a very large coccus isolated from vaccine lymph and having forms similar to many of the vaccine bodies and (4) the tiny bacillus and (5) the large cocco-bacillus spoken of in the first part of the paper.

From the study of the corneal sections it was shown that:

1. The epithelial cells of the cornea may be phagocytic for leucocytes, red blood cells certain bacteria and variously sized and shaped, chromatin-staining bodies, the so-called vaccine bodies.

2. Apart from the above inclusions certain degenerative changes may appear in the epithelial cells.

3. All of these appearances are most frequently seen in the perinuclear zone.

4. The "vaccine bodies" appear regularly after inoculations of active vaccine or variolous lymph; they do not appear after inoculations of any other substance tried.

The principal observations from these studies may be summarized as follows:—

1. The specific virus of vaccinia grows readily in

the corneas and the skin of rabbits, producing in the latter situation the typical eruption.

2. Ascitic-broth containing the epithelial cells from corneas which have been inoculated with old active vaccine lymph produces the typical eruption of vaccinia, though no organisms can be demonstrated in the ascitic-broth mixture.

3. Such corneas show as the only constant, characteristic appearance, and so-called "vaccine bodies."

4. Vaccinia is not due to any organism or combination of organisms growing readily in ascitic-broth.

Discussion.

Dr. Ewing: The contribution just read shows evidence of such a volume of work conducted on such a judicious plan that I feel that Dr. Williams and her colleagues are to be congratulated upon it. It was a specially important result that Dr. Williams was able apparently to demonstrate an increase of the virus in ascitic broth to which infected corneas had been transferred. Regarding the isolation of various bacteria from vaccine and variola, it is to be noted that we are in the habit of casting aside as unimportant cultures which are occasionally encountered. It may well be that some of these demand further attention, and although previous work in this field has been extensive, it still remains important to thoroughly test as many of these species as possible. The great difficulty of determining the presence or absence of specific effects from such bacteria has led to many erroneous claims for bacteria occasionally occurring in vaccine and variolous exudates; *e. g.* Martin's, Nakanishis', etc.

I had some experience with these difficulties with a bacillus, which I called Bacillus 19. It resembled in

morphology and in the fluidification of blood serum, that described by Martin in 1893. Dr. Park kindly loaned me a calf with which to test this bacillus. Vaccination with Bacillus 19 was negative, and a culture was injected subcutaneously, producing an abscess from which after two weeks the germ was recovered pure. The first attempt to vaccinate this calf with active vaccine failed, but a subsequent vaccination made very thoroughly produced a "dry take." From this "dry take" a second calf was vaccinated also with an unsatisfactory "dry take." Both of these calves were vaccinated the second time and each gave as good a take as after the first vaccination. These results on the calves being unsatisfactory, the bacillus was finally set aside when it was proven inert on monkeys.

I was also interested in Dr. Williams' observations which favored the belief that red cells might become transformed into vaccine bodies.

PRELIMINARY REPORT OF EXPERIMENTS WITH FILTERED VACCINE.

WM. H. PARK, M.D.

As is well known, attempts to produce vaccination with the fluid which had passed through a Chamberland or Berkefeld filter have uniformly failed. The present experiments were undertaken with the idea of using crushed virus and of concentrating the filtrate. It was thought to be conceivable that the vaccine organisms might be small enough to pass through the filter and still, because of being contained in the epithelial cells, they might not pass, the cells themselves being held back.

It was also thought possible that the filtrate might contain a few vaccine organisms, and yet have an insufficient number to cause a vaccination. If this condition existed, it seemed possible that by quickly evaporating the filtrate, sufficient concentration of virus might take place to produce vaccination. The following experiments were tried:—

Fresh virus was dried over sulphuric acid in a vacuum. This vaccine material was crushed up with fine sand, using a pressure of twenty-five tons to the square inch. This material was rubbed up with water and separated into two portions. One was drawn through a Chamberland, the other through a Berkefeld filter. Both filtrates were evaporated over sulphuric acid in a vacuum. Calves and rabbits inoculated failed to take. Similar virus was crushed with the sand twice and again six times. The filtrate from this material failed to take. Ordinary glycerinated vaccine and active serum from calves were filtered and failed to take in calves or rabbits. All these calves were kept in a special building belonging to the Department of Health where no possible contamination could occur.

The calves treated in the same way in the vaccine building developed a few typical vesicles, but these are believed to have been due to contamination from the other calves and not to substances which passed through the filter.

THE RELATION OF STREPTOCOCCUS PYOGENES TO VARIOLA.

JAMES EWING, M.D.

From the routine bacteriological examination by ordinary methods of the cadavers of twenty-nine cases of

small-pox, the streptococcus pyogenes was demonstrated in the heart's blood in all cases, and in the cutaneous lesion of about ninety per cent of the cases. This result with the heart's blood is only in accord with the results of some previous studies, as Le Dantec (*Arch. de Med. Navale* T. 64, p. 410) obtained the streptococcus in the heart's blood of fifty consecutive cases.

The abundance of the growth was uniformly proportional to the severity of the disease, and in the primary hemorrhagic cases the cultures were remarkably profuse.

In two cases the streptococcus was associated with the Klebs-Loeffler bacillus, in subjects exhibiting extensive tracheal diphtheria; and in two cases dying late in the disease, with much ulceration of the skin, staphylococcus aureus also grew. Once a special bacillus, called Bacillus 19, was also obtained. In five cases, cultures of ten c.c. of circulating blood were examined with a negative result. Only one of these cases was fatal.

Arnaud, however, reports finding the streptococcus in the circulating blood in two primary hemorrhagic cases (*Rev. de Med.* 1900, p. 303). The demonstration of the streptococcus in the cutaneous lesions in nearly ninety per cent. of these cases indicates the very general presence of this organism in these lesions. Perhaps more careful technique would have given more uniform results, as chain cocci were twice found in sections of the skin when cultures were sferile.

Most of the autopsies were performed within one to six hours after death, and while there was opportunity for post-mortem development in some cases examined after a longer interval in hot weather, the usual abundance of the streptococcus did not fail in early autopsies in very cold weather.

The uniform presence and especially the remarkable abundance of the streptococcus in the cases seems parallelled in no other conditions except in scarlatina and puerperal septicaemia, while the histological lesions indicate widespread effects of the growth of this micro-organism similar to those of pure streptococcus infection. This is especially true in the primary hemorrhagic cases in which the bacteriological and histological study of the cadaver strongly indicates that the streptococcus overshadows any other micro-organism that may be present.

These conditions have led me to conclude that it will always remain impossible to reproduce the fulminant type of variola without the aid of this micro-organism.

When the streptococcus is present in the circulating blood in enormous numbers and on the second or third day of a disease, the question naturally arises whether the presence of this micro-organism in the disease should be classed as a simple secondary infection. Nevertheless, the known facts do not warrant the assumption of a necessary symbiosis because vaccine produces specific lesions when no streptococci can be demonstrated in the virus. The condition seems very nearly like those described by Schleich as "syntoxic parasitism," although present indications favor the belief in a micro-organism too small to be seen with the microscope rather than in the purely chemical nature of the specific virus of small-pox.

A further review of clinical features of small-pox demonstrates other interesting relations of the streptococcus to the disease. In two cases, general scarlatiniform eruptions developed during the course of ordinary discreet variola. One of the cases died in the children's ward in which no further cases of this sort developed. The other went through the usual course of scarlatina

with profuse scaling desquamation, but later developed multiple abscesses in the skin. These cases were, therefore, probably not true scarlatina, but examples of the scarlatiniform septic eruption due to the streptococcus which was abundantly present in both.

Three cases of ulcerative endocarditis were encountered with the usual abundance of streptococci in the heart's blood. One of these gave cardiac symptoms from the first and died on the eighth day with extensive ulceration of the mitral valve and a rather scanty eruption.

Finally, erysipelas and diffuse phlegmonous inflammation of the skin are very common incidents in small-pox hospitals.

In these various clinical features of the disease the streptococcus exhibits its well-known capacity to produce a great variety of specific clinical effects, and in them it seems to follow the ordinary rules governing secondary infection. In these clinical forms of the disease it is interesting to note how this micro-organism can multiply in the system; first, as a complicating factor in the cutaneous lesion of small-pox; then as the excitant of a scarlatiniform eruption, then, under the form of pyemic abscesses, or as ulcerative endocarditis or erypsipelas, etc., while none of these manifestations seem to confer any immunity to the subsequent attack of the micro-organism in another clinical from.

Discussion.

Dr. Park: Dr. Ewing has demonstrated that in a large series of fatal cases streptococci were universally present in the blood and in the pustules of the skin aud mucous membranes. It seems to me he goes too far, however, in thinking that the streptococcus is

necessarily present before the symtoms and lesions which we find in small-pox can develop. Both scarlet fever and small-pox cause marked changes in the mucous membranes of the throat. Here we have the streptococci regularly present. The profound changes produced by the poison of variola combined with the location of the streptococci seem to me to account for the secondary infection of the streptococcus. If no streptococci existed, I believe we would still have severe and probably fatal cases of small-pox.

Dr. Ewing: My own ideas regarding the relation of the streptococcus to variola are by no means clear, but I have been greatly impressed with very early appearance and enormous development of the streptococcus in the disaase. Its behavior in the primary hemorrhagic cases and in some other forms seems different from that of an ordinary secondary infection. I do not find either that the term "symbiosis" is justified by the facts. It would seem that the small-pox virus annihilates resistance to the growth of the streptococcus, with results which occasionally resemble fulminant puerperal septicæmia, where the streptococcus is the only micro-organism present. Perhaps Schleich's term "syntoxic parasitism," most closely expresses what seems to me to be the condition in primary hemorrhagic small-pox.

As to the relation of red cells to vaccine bodies, I am not prepared to claim that all vaccine bodies are derivatives of these cells, but having found that in the tissues the fragments of red cells are a very common origin of vaccine bodies, I am inclined to think that all vaccine bodies may have the same origin. In the cornea it seems almost hopeless to attempt to prove this origin, in which case the theory of the exclusive origin of vaccine bodies from red cells must fail of demonstration. I have

already found that red cells are present in the inoculation wounds of the cornea under somewhat surprising conditions. Therefore, considering all the evidence against the parasitic nature of vaccine bodies, at a time when some Continental observers are grouping carcinoma and variola together, it seems justified to urge that some and possibly all of the appearances on which this relation is based are referable to red cell detritus.

THE CHEMICAL THEORY OF SMALL-POX.

W. S. MCGILL, M.D.

To rehearse before the members of this Society the history of our present etiology of variola would be for the most part but wearisome repetion if reviewed in full. With your permission then, I will allow myself to pass rapidly over the multiplicity of details.

Let us recall that, in small-pox we have one of the most ancient of the eruptive plagues, the longest known of contagious diseases, and the pioneer in our conception of immunity as a possibility long before its rational basis and fundamental components were even thought of.

More than two centuries ago it was a well credited fact that small-pox was an infectious disease, The strict limits of its contagion have been drawn more closely and more insistently urged with each advancing step of epidemiology; but only gained their firm establishment with the advancement of our knowledge of bacteriology.

In the first glow of germ discovery, the bacterial origin of all infectious diseases was widely proclaimed and still more generally credited. The numerous announcements of the specific coccus, bacillus or other bacterial origin of variola have not yet reached the end of

the list, nor the final withdrawal of their rival claims; but their very numbers have succeeded in creating a widespread scepticism as to their scientific value and a strong insistence that no further immature investigations be brought out.

With a full realization of the benefit of working in times of such prevalling scepticism, it is particularly advantageous to examine very carefully the grounds on which a claim for the bacterial cause of small-pox can rationally be placed.

Remember the length of time during which this disease has been particularly distinguished as due to a *specific virus*, its possibilities of attenuation, inoculation, vaccination and resultant and acquired immunities, communicability from human to animal races and reciprocal attenuating action.

With the exception of the determination of a specific organism, its culture in vitro, and subsequent inoculation to reproduce the clinical form of this specific disease, we have had for years, in variola, a typical picture of a bacterial infection of the human being. With the exception of the results of the work of Bosq and Roger, to be considered in a moment, bacteriology has, by the failure to identify the specific germ in strict accord with its well-known rules for such scientific investigation, no claim to include in its family the determining agent of variola.

The close resemblance of the symptomatology of variola to that of typical bacterial infection of man, makes it easy to understand that the first move of the incipient promulgator of a theory of such infection would turn to variola; to find there most easily the requisite bacteriological proofs. What has been the result of his inquiry? Thirty years of close investigation, in some cases, by the best bacteriologists of the world, have to-

tally failed to demonstrate any form of bacterism entitled to the slightest claim of specificity.

Nevertheless, the facts are fully demonstrated (without denying the possibility and frequence of a secondary bacteriological infection of the variolic pus) that variolic fluid is often found, containing no bacteria discoverable by any known method of stain or culture. Such fluid, often pus, will nevertheless invariably reproduce the specific disease when inoculated to the proper receptive medium. These positive inoculations of material absolutely deprived of bacteria have been made: in case of a hemorrhagic variola, the blood drawn from the vein at the elbow, one hour before death; again, with material from certain visceral lesions, in which the total absence of bacteria was assured by multiplicity of tests of stain and culture; and also by inoculation of similarly verified, bacteria free, sero-purulent liquid, obtained from the vaginal tunic during the course of variolic orchitis.

It has been amply demonstrated elsewhere that such pus and the blood of hemorrhagic cases inoculated into animals may be continued in series, inducing a subsequent immunity to variolic inoculation and in some, provoking death.

Blood from a case of hemorrhagic variola has for a long time been carried in a continuous series of animal inoculation and re-inoculation, at the Carnegie Laboratory, and invariably provokes a fatal disease, in our chosen animals, and of a determined type with persistence of characteristic lesion. What we are gathering are the data to prove it to be the specific agent of small-pox.

To prove the bacteriological origin of variola, it is but natural to attempt to introduce another cause. The protozoon is thus advanced; first by Renaut, in 1881, followed most prominently by Van der Loeff and Pfeiffer.

The first to make the subject of a protozoic etiology the question of direct experiment seems to have been Guarnieri.

Numerous workers since that time have very well established that in the various organic liquids of a variolic subject and in vaccine, also in certain of the tissue cells, noticeably the epithelial, and producible there by the inoculation of variolic pus or vaccine are small, characteristic elements, the so-called variolic bodies. To properly interpret the nature and significance of their existence constitutes a very important point of discussion in any consideration of variola at the present time.

The conclusion of the question whether these minute corpuscles are parasites or simply derivatives of tissue cells does not appear to be furthered by the confinement of experiment to the inoculation of corneal epithelium. For several investigators, myself included, report the irregularity of corneal reaction even to known virulence of the inoculated material. Moreover, the bodies there found and frequently offered as typical so-called variolic bodies, have been produced by experiments of purely traumatic nature. The exhaustive study, by Ranvier, of the special tendencies of the epithelial cells of the cornea to heal a traumatism in such peculiar manner as to make inevitable an accumulation of both extra- and intra-cellular minute staining bodies, known to be of nuclear debris origin, would seem to make it imperative upon the conscientious experimenter to choose some other epithelium than this for decisive investigations of a question absolutely depending upon the elimination of a possibility of cell-debris being mistaken for parasites. Without entering upon the question of debris *vs.* parasite as it should be considered, it can, I think be fairly stated that many de-

monstrations of the so-called bodies in the cornea were probably only cell-debris. It is my own personal opinion that such is the real origin of all of the corneal bodies, that it has been my opportunity to examine. In defense of my opinion may I be permitted to add that experiments to invalidate the proposition of cellular residues should not be executed in that particular point of epithelium (the corneal) which Ranvier has so explicilty shown, inevitably heals its wound by a process, peculiar to that tissue and of necessity depositing at the locality of the traumatism, a mass of disrupted cell components, even nuclear fragments.

Returning from the digression, let us resume that, the question at issue is in substance: first, are these minute corpuscles parasites or derivatives simply of re-existing tissue cells or to further elaborate, are they nuclear debris resulting from the death or possibly chemical disruption of the cells, or as Dr. Van Gieson puts it in tentative suggestion; are these degenerative or atavistic cellular elements resultant from the changed chemical or physiological action of the surroundings, or produced by the invasion of a parasite, or are they the adventitious or specific organisms themselves?

The line of investigation to determine this question has been long varied and thorough. Some of it admirably true to the scientific method of research, but possibly through slight lack of system, perhaps not receiving the notice it deserved, seems to have failed to attain the goal as rapidly as it promised.

Some of the reports of the small-pox investigations have come out recently at Washington, some you have heard this evening. But even to mention the names and subjects of the workers of the last few years, is beyond the limits of this paper.

Standing out in strong relief from all other recent investigations, are those of Bosq and Roger in France. Here with the most explicit statement of laboratory experiment, are to be found all of the steps of identification of a specific organism. Here is research work in strict conformity with the limits of identification prescribed by Microbiologists. We can but say that the reports of Bosq and Roger seem to have contributed the most important data for the adherents of the protozoic element as the specific microbe of variola.

The variolic bodies are to be found in the blood of hemorrhagic cases before their eruption, and the blood containing them is proven to cause the variolic infection, when drawn from the patient as early as the third and fourth day of the incubation period of the disease. From the first and second day of eruption, up to the stage of suppuration, as the degree of the power of contagion increases in geometrical ratio, the specific elements appear and increase in the hematopoietic organs; and then, in the epithelial membranes (skin und mucosa), papules becoming vesicles and subsequently pustules are found, without the occurrence of the slightest contamination of adventitious bacteria detectable by any stain or culture.

The desquamations, excreta (including the urine in cases of hematuria), all organic fluids and residues reached by the blood of a variolic patient contain the special bodies.

They are exceedingly resistant, and if really the specific element, are proven by direct experiment to survive air-desiccation for at at least two years. They retain an unimpaired power of contagion, after exposure to the intense cold of evaporating liquid air, after subjugation to the hydraulic pressure of 4000 pounds to the square inch, and even after heating for a limited time to the

vicinity of 70 degrees C. The resistance of the germs of variolic contagion to the fumes of sulphur, as usually employed in the disinfection of mail-matter is insisted upon by Roger, and by him it is shown that this survival of the contagion is inevitable, unless the fumes are brought into the most direct and prolonged contact with the sulphur vapor.

The transmission of the specific microbe to the fœtus has twice been demonstrated by actual examination of organs involved. In two cases of abortion, one at the fifth, the other at the sixth month of pregnancy (the mothers suffering with confluent small-pox), the amniotic fluid and the fœtal tissues themselves revealed the presence of the described specific microbe of variola. In fact this connection it may be well to draw attention to the that infection of the fœtus is rarely noted as apparent previous to a delivery during the ninth month of the intra-uterine life. With the increased knowledge, to be derived trom the experimental study and verification of its specific agent, it is recognized that the period of incubation is in animals a somewhat variable quantity and the same is true at the beginning stages of human life. The birth of a child, whose first eruption follows at least 30 days later the first appearance of the maternal infection, is a fact well established. Moreover, it is striking to notice that the pronounced type of acute and anemiating septicemia with variola presents in many of our experiments with animals, accentuates this side of its action as the resistance of the organized animal form is rendered lower in our line of experiments: this septicemic type of variola is strikingly predominant in the fœtus and very young child. The form of variolic infection without the slightest eruption is well known to all students of this disease in the rabbit. The comparatively

frequent occurrence of a like infection is one of the common notes of the clinic relative to new born infants. The possibility of this origin of the so-called natural immunity is here suggested.

In the usual course of infection, the specific organisms penetrate the respiratory tract, where if successful in traversing the bronchial epithelium, it enters the blood, by it is diffused throughout the organism, in it lives and multiplies, producing the well known symptoms of variola; a veritable general toxemia, of which the eruption is the mere epithelial reaction or method of excretion, as subsequent investigations may determine.

The juices of the digestive tract destroy the parasite. They are likewise incapable of penetrating the intact cutaneous epithelium, but invade rapidly the slightest denudation of that surface.

According to the numbers of the originally infecting organisms, the depth of the cellular lesions produced is variable. The blood is always profoundly altered, marked by a characteristic mononucleosis, the discovery of which is considered sufficient evidence on which to base a doubtful diagnosis.

To prevent the danger of extending this paper beyond its time allowance, it is necessary to turn aside in order to present to you, an outline of chemical study of the question.

In order to more exactly outline the determinant factors of the variolic symptomatology, as well as the bearing of such factors on the etiology, I have devoted a considerable amount of time and effort upon purely chemical considerations of variola.

The results of that work have surpassed my expectations in some respects. They have also brought me face to face with many difficulties to such an extent that

it is now only possible to mention some of the lines and the partial development so far attained.

The chemical study was most strictly confined to the vaccine matter furnished by Dr. Park, from the Research Laboratory of the Health Department of the City of New York.

The samples were of pure vaccine pulp, as scraped from the calves, and also from the prepared (glycinerated) pulp. Every sample was carefully analyzed for matter of alkaloidal nature and in two cases were positive in results.

By processes of multiple solvents and separations, in both of these cases, it is found that we have to deal with an hitherto unidentified alkaloidal substance. Portions of the most purified material to be obtained are to undergo ultimate organic analysis as soon as possible. From every sample of vaccine matter were obtained diastasic material, but of great variability of composition and properties. Among them are apparently three distinct types of diastase. There is one which slowly but directly acts upon the protein matter, one which is very stupefying to the experimental animal when injected intravenously, but does not seem to retain any permanent effect. In this connection, I must add however that no observation of the phenomena of nutrition were made in these experiments; and these would seem to be very imperative, with a view to determining the extent of chemical influences in provoking the anemic and wasting phenomena of the sub-acute type of disease which variola itself reveals in the animal.

The third type of diastase isolated from the vaccine samples was most peculiar. Injected intravenously into rabbit and guinea-pig, it provoked a very rapid and startlingly violent convulsion. A prelude of fibrillary tremor,

collapse to one side resulting in the fall of the animal; this was followed by periodic muscular twitchings of less and less violence, a final repose and ultimate recovery in from thirty to forty minutes. Further experimentation of this diastase was impossible by reason of the limited supply of the material. And constant attempts to find it in another sample have thus far failed.

As you will easily realize the short time at our command thus far in work of such nature has permitted the collection of a number of these peculiar chemical substances; but has given us, as yet, very little time for the study of their nature. We must go very slowly, for it is untrodden ground, and our material is very limited in quantity and hard to obtain. It seems to be an exceedingly difficult problem to find any two of these substances which are exactly alike.

In order that the bearing of such investigations be understood will you permit me to call your attention to the increasing realization of the predominant role of the chemical problems in majority of our infectious diseases. The questions of immunity, serumtherapy, and scientific vaccinations depend upon them. It is a tenable position to take that the tissue lesions, the cellular and nuclear debris, the degeneration and atavistic retirement of cells, about which so much is said, are most directly resultant to the purely chemical influences of the affected tissue's surroundings.

To determine the proper sphere of influence of the chemical forces in question is the scope of our chemical investigation of the variola problem. Its difficulties can be no proper deterrent, for the importance of its determination is pre-eminent. There lie the secrets of the true pathology of so septicaemic a disease as this; and

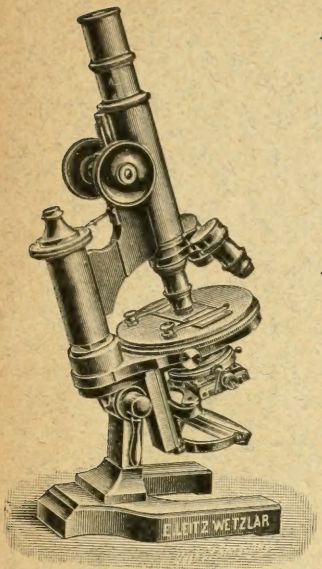
there, too, are to be found the facts which will permit the realization of rapid and real immunity.

Regretting that I have not the time, and perhaps this is not the place, for presentation of such chemical investigations in detail, it remains to close with the statement of conclusion that the ferment substances of vaccine matter are extremely alterable, limited in activity and amount when compared to the like substances of the variolic tissues. Consequently, the more promising field for such chemical studies is to be found in freshly infected variolic tissues. These are now being used and already show less variability.

DEMONSTRATIONS OF THE SKIN LESIONS OF SMALL-POX.

S. POLLITZER, M.D.

A remarkable and most instructive series of lantern slides of the gross and microscopical lesions of variola were demonstrated by Dr. Pollitzer.



Microscopes
Microtomes
AND
Accessories

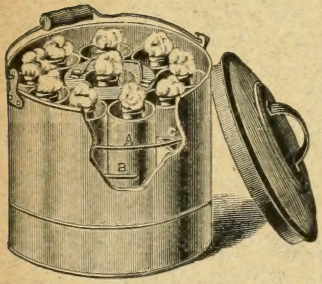
NEW
LARGE FOLDING
MICROSCOPE

Catalogue sent on application

NEW YORK
411 West 59th St.

E. LEITZ,

CHICAGO
659 W. Congress St.



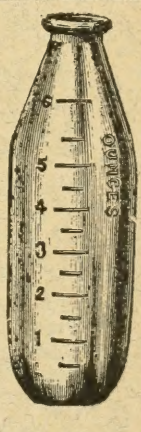
FAMILY SIZE

PASTEURIZER

as

Devised by Dr. Freeman

Bottle of Improved Form for Pasteurizing and for Feeding.



Bacteriological and Laboratory Supplies

INCUBATORS, HOT AIR OVENS,
GLASSWARE, STAINING FLUIDS, Etc.

JAMES T. DOUGHERTY, 409-411 West 59th St., N. Y.
Branch Store: 334 East 26th St., N.Y.